Reproduction-Induced Changes in Maternal Bone Confer Protective Effects against Estrogen Deficiency

Chantal de Bakker, BS
Laurel Leavitt
Casey Krickus
Wei-Ju Tseng, MSE
Tiao Lin, MD
Wei Tong, MD
Ling Qin, PhD
X. Sherry Liu, PhD

Introduction

Pregnancy and lactation induce substantial maternal bone loss, which undergoes a partial recovery post-weaning[1]. However, even after a lengthy post-weaning period, permanent alterations in the maternal skeleton remain[2,3]. At the same time, clinical studies demonstrate that reproduction does not increase future risk of osteoporosis or fracture[4,5]. To explain this paradox, we hypothesized that the permanent skeletal changes induced by reproduction may confer protective effects against postmenopausal bone loss. To test this hypothesis, we tracked changes in bone structure at the proximal tibia following ovariectomy (OVX) in virgin rats and in rats that had undergone 3 cycles of pregnancy and lactation.

Methods

Animal Protocol

All experiments were IACUC approved. Female, Sprague Dawley (SD) rats were assigned to two groups: Reproductive (n = 9) and Virgin (n = 4). Starting at age 3 months, reproductive rats underwent 3 repeated cycles of pregnancy and lactation, with a 6-week post-weaning recovery period between each cycle. At age 12 months, all rats underwent OVX surgery to induce estrogen deficiency, and their proximal tibiae were scanned by *in vivo* μCT prior to surgery, as well as 12 weeks post-OVX (10.5μm, vivaCT 40, Scanco Medical).

μCT Image Analysis

μCT scans made 0 and 12 weeks post-OVX were registered to ensure a consistent trabecular volume of interest (VOI)[6], and trabecular bone volume fraction (BV/TV), connectivity density (Conn.D), trabecular number (Tb.N), and trabecular thickness (Tb.Th) were measured. To evaluate whether variations in baseline microarchitecture can impact the bone loss rate, linear regression analysis was performed, whereby baseline trabecular parameters were correlated to the % decrease in BV/TV following OVX. Individual trabecular dynamics (ITD) analysis[7] was performed to evaluate the rate of structural deterioration (defined as the number of instances of plate perforation and rod disconnection). Cortical bone structure at the proximal tibia, including cortical area (Ct.Area), cortical thickness (Ct.Th), and polar moment of inertia (pMOI) were evaluated, and whole-bone stiffness was estimated through finite element analysis (FEA).

Results

Trabecular Microstructure

Over 12 weeks post-OVX, virgin rats underwent 76%, 86%, and 50% decreases in BV/TV, Conn.D and Tb.N, respectively ($p < 0.05$) with no change in Tb.Th (Figure 1). In contrast, reproductive rats showed a 53% decrease in BV/TV, with no changes in Conn.D, Tb.N, or Tb.Th. Prior to surgery, reproductive rats had 43%, 73%, and 46% lower BV/TV, Conn.D, and Tb.N, respectively, than virgins ($p < 0.05$), but by 12

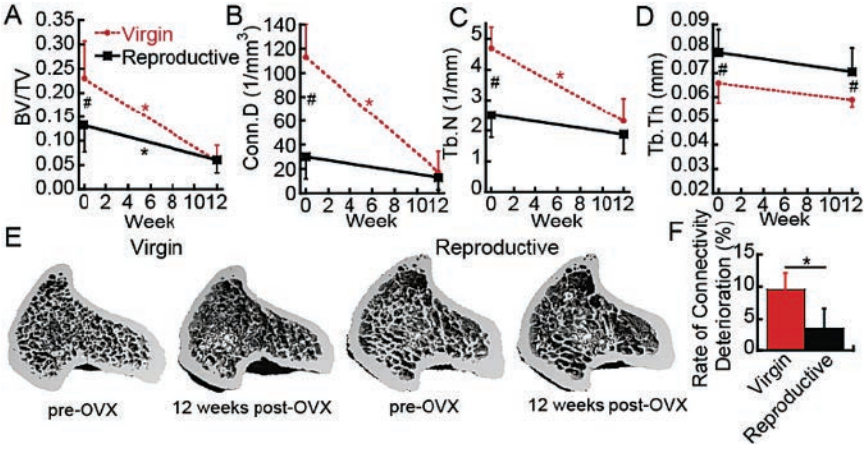

Figure 1. (A-D) Longitudinal changes in BV/TV, Conn.D, Tb.N, and Tb.Th at the proximal tibia. •: wk12 ≠ wk0 ($p < 0.05$); #: Virgin ≠ Reproductive at wk0 or wk12 ($p < 0.05$). **(E)** 3D renderings of the proximal tibia of a virgin (left) and reproductive (right) rat pre- and post-OVX. **(F)** ITD-based rate of connectivity deterioration (defined as the rate of rod disconnections and plate perforations) post-OVX.

weeks post-OVX, these parameters were no longer different between the two groups. Reproductive rats had 20% elevated Tb.Th relative to virgins throughout the study. These results were confirmed by ITD analysis, which illustrated that virgins underwent a 167% higher rate of structural deterioration than reproductive rats.

Correlation of Baseline Structure and Degree of Bone Loss

Linear regression indicated that baseline BV/TV was not significantly correlated to the % reduction in BV/TV post-OVX. However, baseline Conn.D, Tb.N, and Tb.Th were significantly correlated to the degree of OVX bone loss (Figure 2, $r = 0.72\text{-}0.80$; $p < 0.05$). Baseline Conn.D and Tb.N were also both significantly correlated with baseline Tb.Th, and partial correlation analysis indicated that, after adjustment for baseline Tb.Th, baseline Conn.D and Tb.N were no longer correlated with the degree of post-OVX bone loss, suggesting that baseline Tb.Th was the most important factor explaining the degree of post-OVX bone loss.

Cortical Structure and Whole-Bone Stiffness

Both reproductive and virgin rats underwent no changes in Ct.Area, Ct.Th, or pMOI post-OVX (Figure 3). Reproductive rats had greater Ct.Area, Ct.Th, and pMOI (16%, 20%, and 24%, respectively; $p < 0.05$), than virgins throughout the study. Whole-bone stiffness decreased 21% in virgins after OVX ($p < 0.05$), but showed no change in reproductive rats. At 12-weeks post-OVX, virgin rats had 20% reduced whole-bone stiffness compared to the reproductive group.

Discussion

Results from this study confirm the effects of reproduction on maternal bone, as reproductive rats showed inferior trabecular microarchitecture, but increased robustness of cortical bone, prior to OVX. This agrees with previous findings of incomplete recovery of trabecular microarchitecture after reproduction[2,3] as well as a clinical study suggesting that lactation may increase robustness of cortical bone[8]. Furthermore, reproductive history appeared to cause an adaptive response to OVX-induced estrogen deficiency. Reproductive rats showed a lower degree of post-OVX bone loss, resulting in a similar trabecular microstructure between the reproductive and virgin rats at 12-weeks post-OVX, despite differences between the two groups at baseline. Results of our correlation analysis suggest that baseline Tb.Th may be an important determinant of post-OVX bone loss. Thicker trabeculae may be protective against bone loss, as in thinner trabeculae, the elevated rates of bone resorption are more likely to lead to permanent structural damage, whereas in thick trabeculae, increased osteoclast activity may only cause transient resorption cavities which can be refilled through coupled bone formation. However, further studies are required to confirm this hypothesis. Finally, our study indicated a lower degree of whole-bone stiffness deterioration in reproductive rats than in virgins, suggesting that reproductive history may have a protective effect on postmenopausal bone strength. This was likely a result of the lower degree of post-OVX trabecular bone loss, combined with the greater robustness of cortical bone in the reproductive rats.

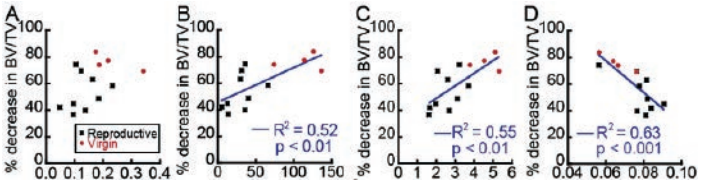

Figure 2. Correlation of the degree of deterioration in BV/TV post-OVX with baseline **(A)** BV/TV, **(B)** Conn.D, **(C)** Tb.N, and **(D)** Tb.Th.

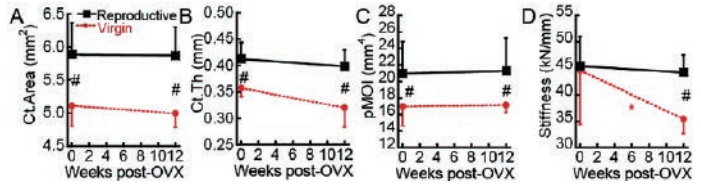

Figure 3. Post-OVX changes in **(A)** Ct. Area, **(B)** Ct.Th, **(C)** pMOI, and **(D)** whole-bone stiffness. •: wk12 ≠ wk0 ($p < 0.05$); #: Virgin ≠ Reproductive at wk0 or wk12 ($p < 0.05$).

Significance

The effects of reproduction on bone health are controversial: reproduction induces irreversible skeletal changes, but does not increase later risk of fracture. This study indicates that the unique phenotype of post-reproductive bone confers protective effects against postmenopausal bone loss.

Acknowledgements

NIH/NIAMS P30AR050950, NIH/NIAMS R03-AR065145, NSF Graduate Student Research Fellowship.

Disclosures: None of the authors have any disclosures relevant to the subject of this work.

References

1. Kovacs CS, Ralston SH. Presentation and management of osteoporosis presenting in association with pregnancy or lactation. *Osteoporos Int.* 2015;26(9):2223-2241.

2. Bowman BM, Miller SC. Skeletal mass, chemistry, and growth during and after multiple reproductive cycles in the rat. *Bone.* 1999;25(5):553-559.

3. Liu XS, Ardeshirpour L, VanHouten JN, Shane E, Wysolmerski JJ. Site-specific changes in bone microarchitecture, mineralization, and stiffness during lactation and after weaning in mice. *J Bone Miner Res.* 2012;27(4):865-875.

4. Melton LJ, 3rd, Bryant SC, Wahner HW, *et al.* Influence of breastfeeding and other reproductive factors on bone mass later in life. *Osteoporos Int.* 1993;3(2):76-83.

5. Paton LM, Alexander JL, Nowson CA, *et al.* Pregnancy and lactation have no long-term deleterious effect on measures of bone mineral in healthy women: a twin study. *Am J Clin Nutr.* 2003;77(3):707-714.

6. Lan S, Luo S, Huh BK, *et al.* 3D image registration is critical to ensure accurate detection of longitudinal changes in trabecular bone density, microstructure, and stiffness measurements in rat tibiae by in vivo microcomputed tomography (muCT). *Bone.* 2013;56(1):83-90.

7. Altman AR, de Bakker CM, Tseng WJ, Chandra A, Qin L, Liu XS. Enhanced individual trabecular repair and its mechanical implications in parathyroid hormone and alendronate treated rat tibial bone. *J Biomech Eng.* 2015;137(1).

8. Wiklund PK, Xu L, Wang Q, *et al.* Lactation is associated with greater maternal bone size and bone strength later in life. *Osteoporos Int.* 2012;23(7):1939-1945.